Why Transgenics are Imperative for Biofuel Crops

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Rehovot, Israel

NASA-Cleveland
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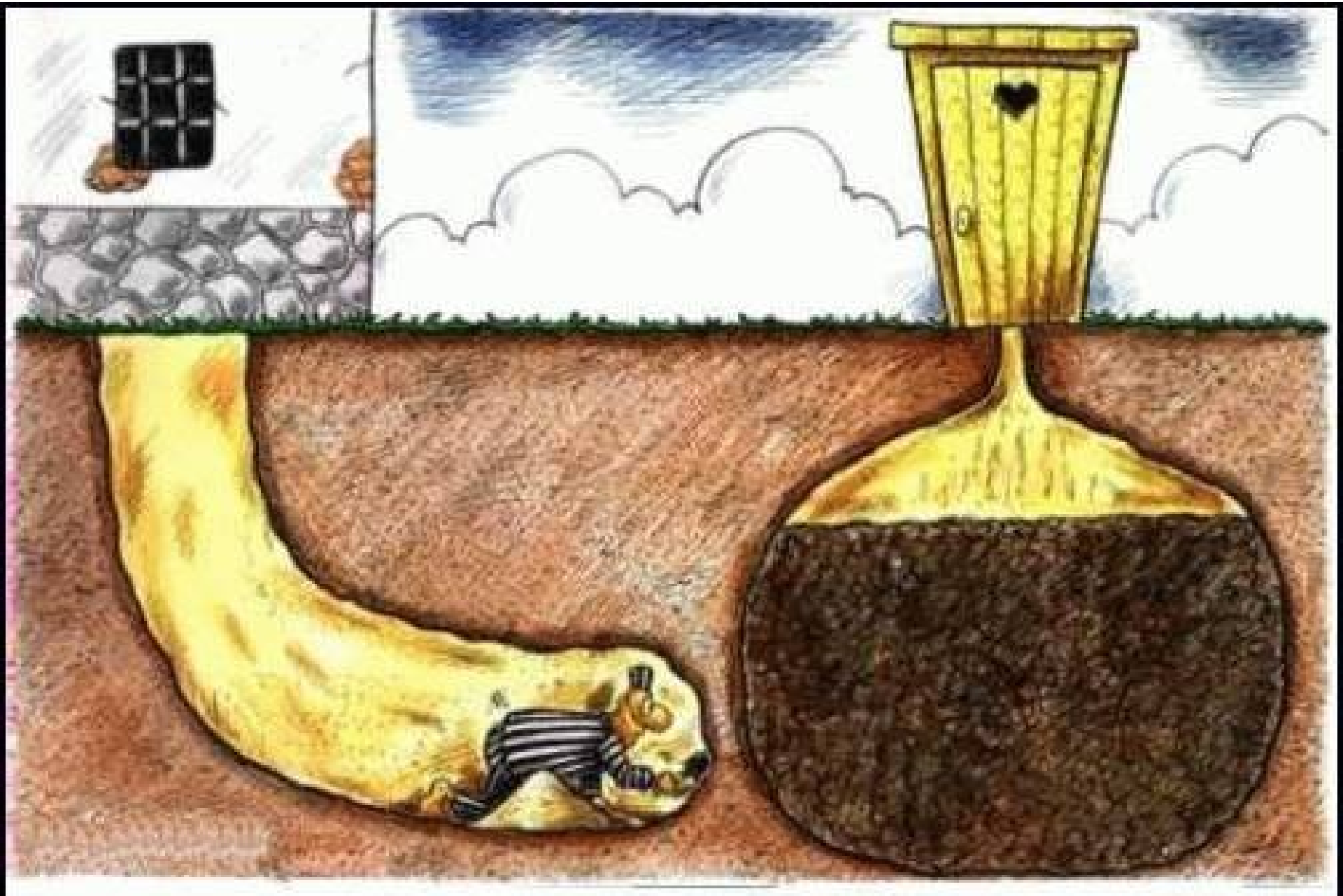
Thesis: No independence with biofuels



Traditional biofuels

← India

Africa ↓

Inefficient
Polluting
Environmentally negative

Can we do better?

In temperate areas the traditional biofuel was oats:

Cultivated on ca. 20% of land

Oats fueled all of farming

Fueled. mules, horses and laborers

Claim: Biofuel crops for marginal lands

If language is incorrect,
then what is said
is not what is meant;
if what is said
is not what is meant,
then what should be done
remains undone

Confucius

If we call land "marginal", will the economic and environmental impact studies that should be done, remain undone?

Do we mean "marginal" only economically?
Do we mean "marginal" only agriculturally?
Do we mean "marginal" only environmentally?

What was on the marginal land?

- another crop
- forest
- wetlands
- grazing land
- wildlife habitat

What are all the implications of changing to biofuel crops?

Deutsche Welle

Energy | 23.04.2007

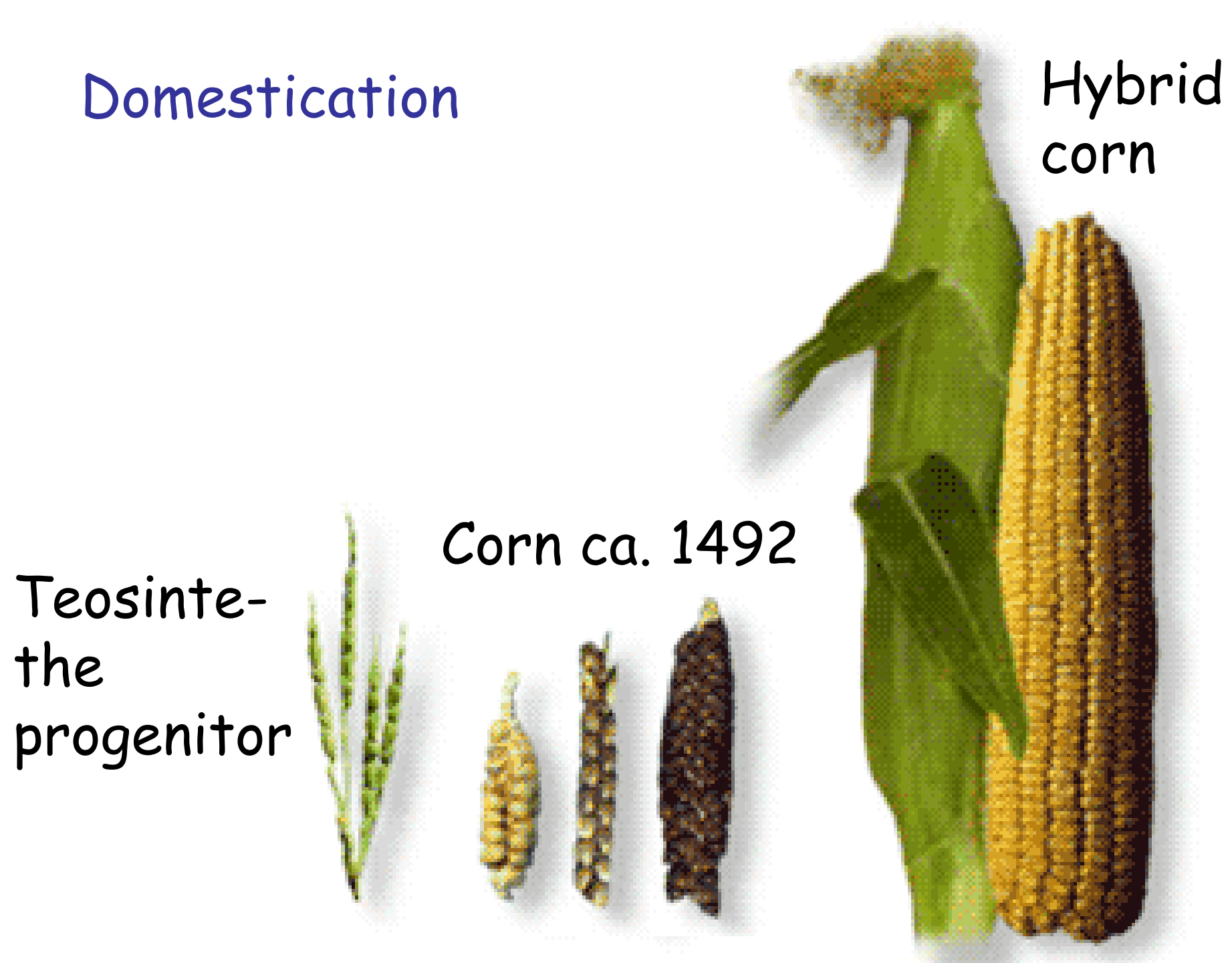
Germany's Cheap Beer Tradition Under Threat From Biofuels

The popularity of biofuels is affecting the price of Germany's most cherished beverage

Germans will have to dig deeper in their pockets to enjoy their beloved beer in the next few months as barley is increasingly displaced in the country's fields by heavily subsidized crops used for biofuels.

Domestication

Hybrid corn

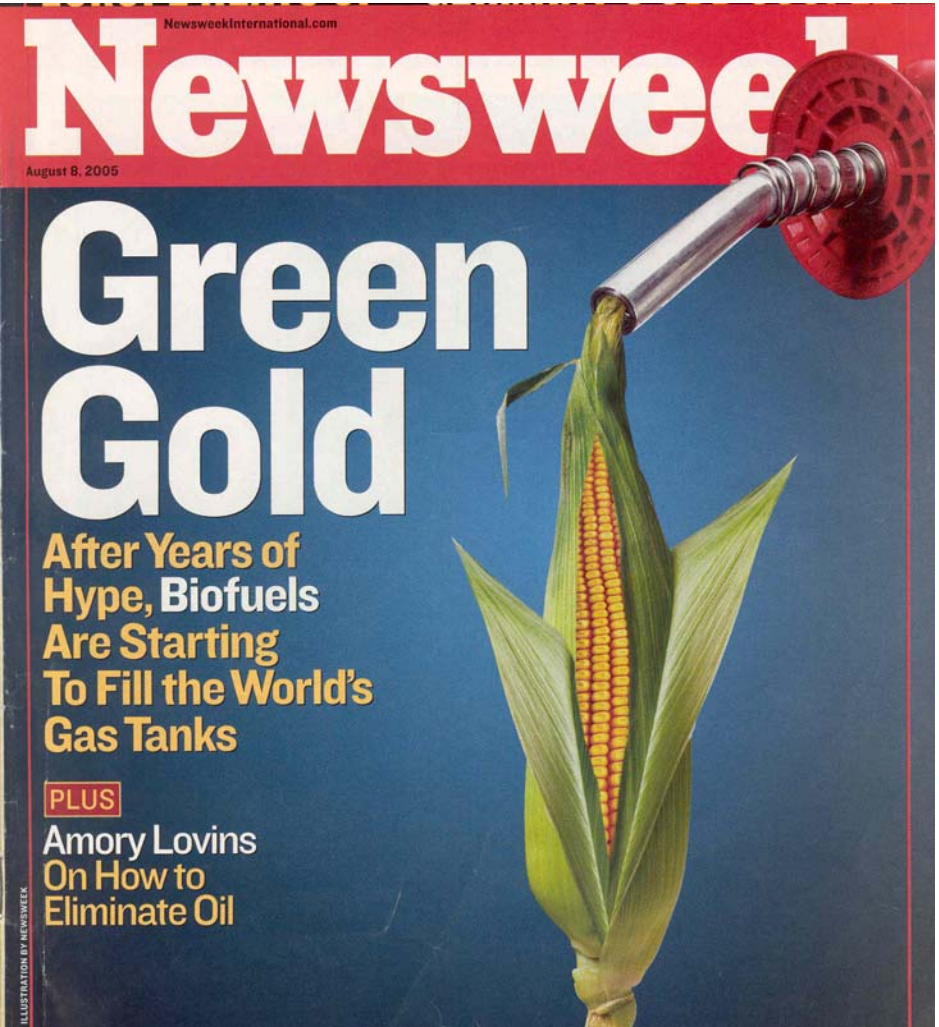


Corn ca. 1492

Teosinte- the progenitor

"New" opportunities:

Where will we get food and fuel with the available land?



Biofuel possibilities:

2nd generation

- grow special crops
- use wastes (straw)

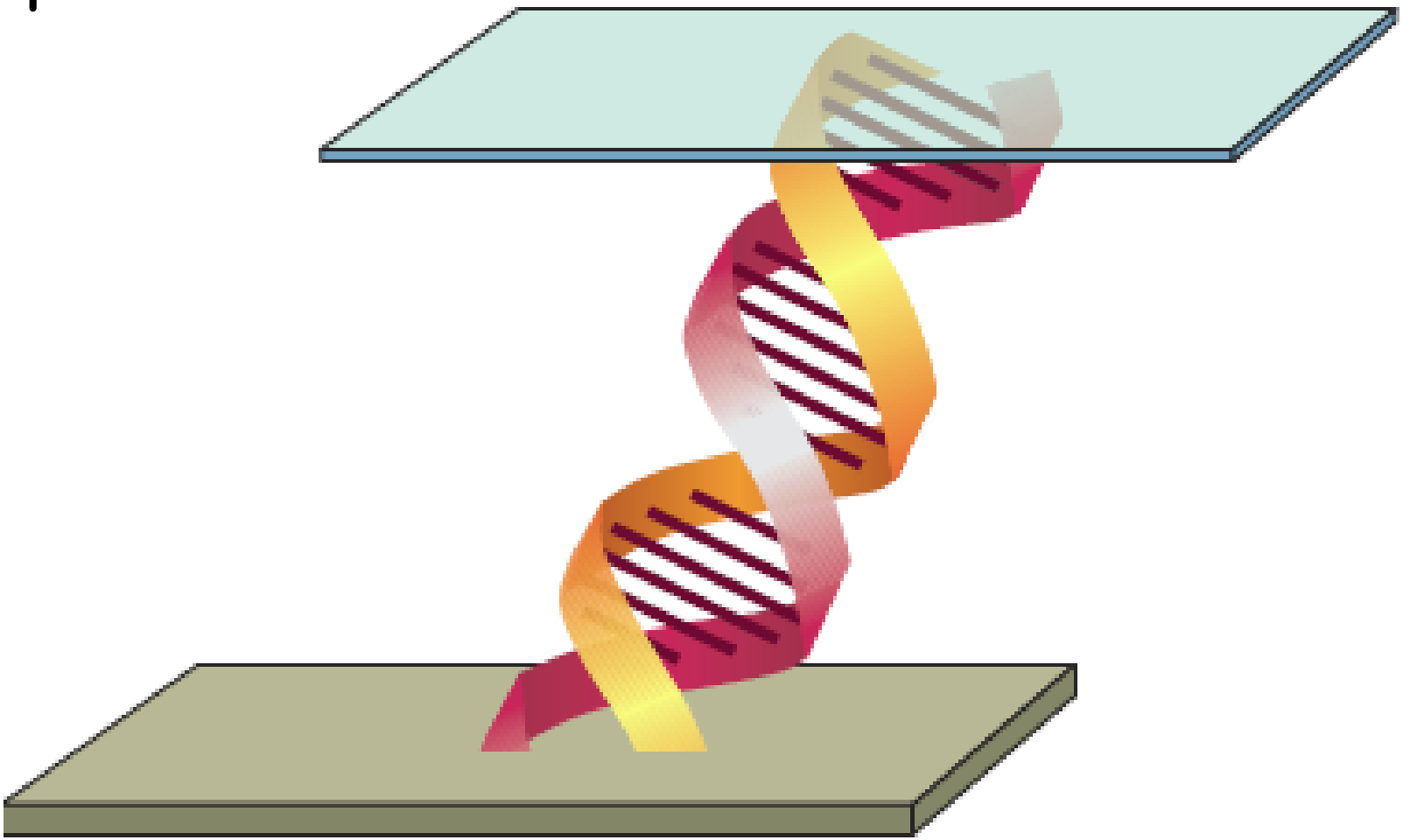
3rd generation

- use algae as crops

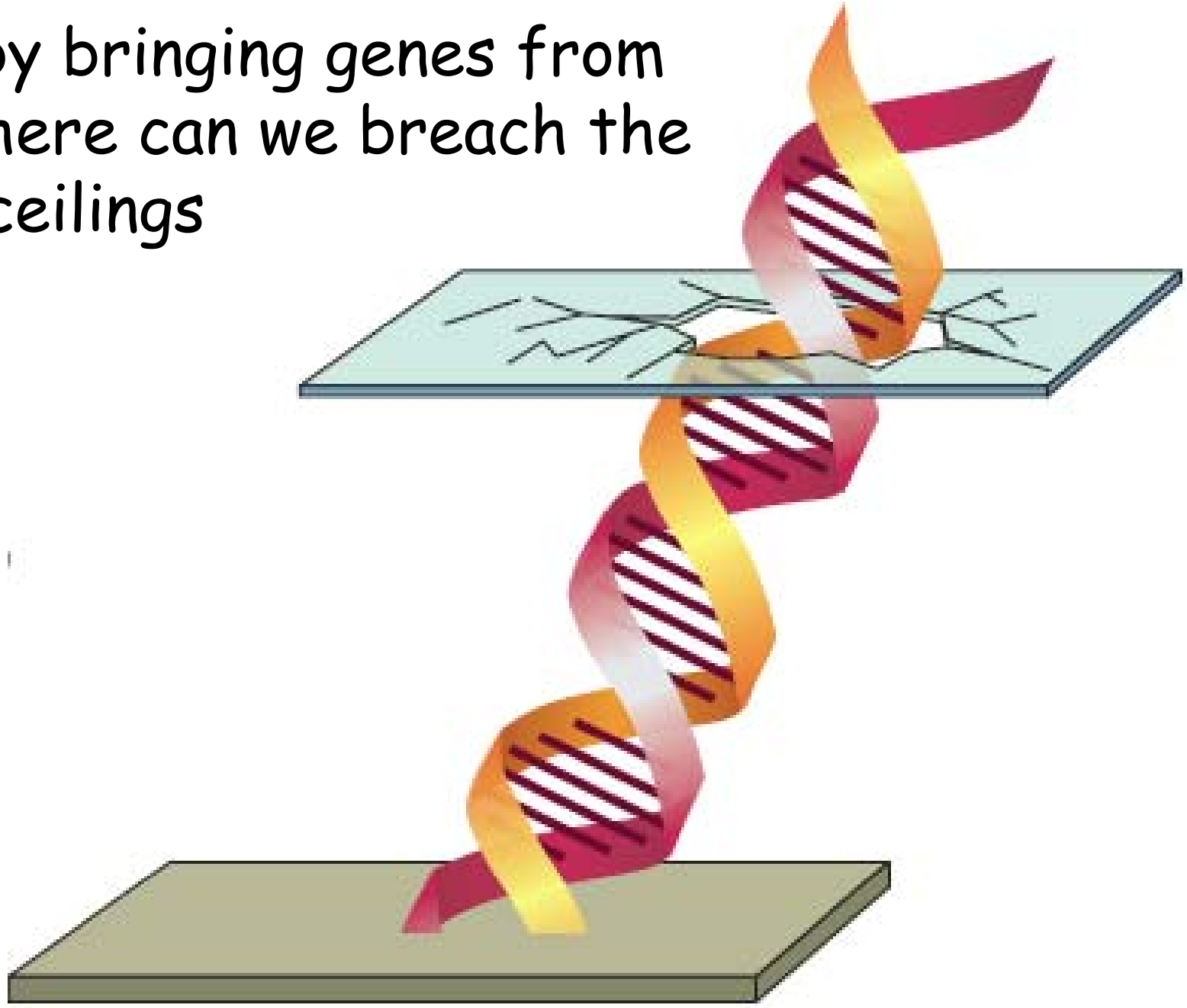
What to do? These crops have not been domesticated for biofuels

The ability to adapt is the function of DNA

You can reach a "genetic glass ceiling" and further recombination by breeding does not help

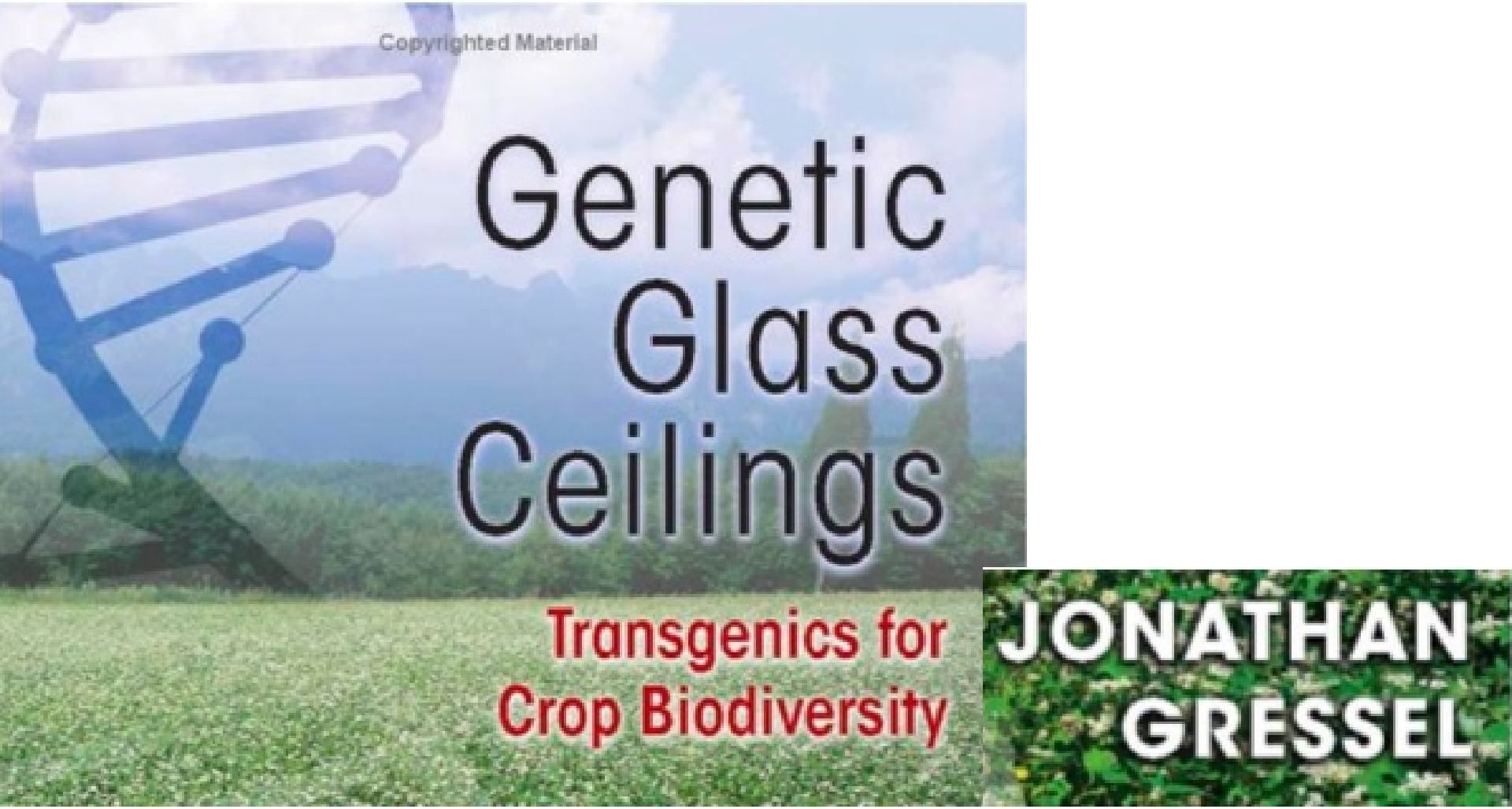


Only by bringing genes from elsewhere can we breach the glass ceilings



To get rapid domestication of biofuel crops

A new book



dealing with further domesticating under-domesticated crops - including biofuel crops

Analyzing and proposing where to get genes

Can the yield barrier be breached?

Sugarcane breeders reached an asymptote

Wu & Birch - Plant Biotech J 5:109, 2007 engineered

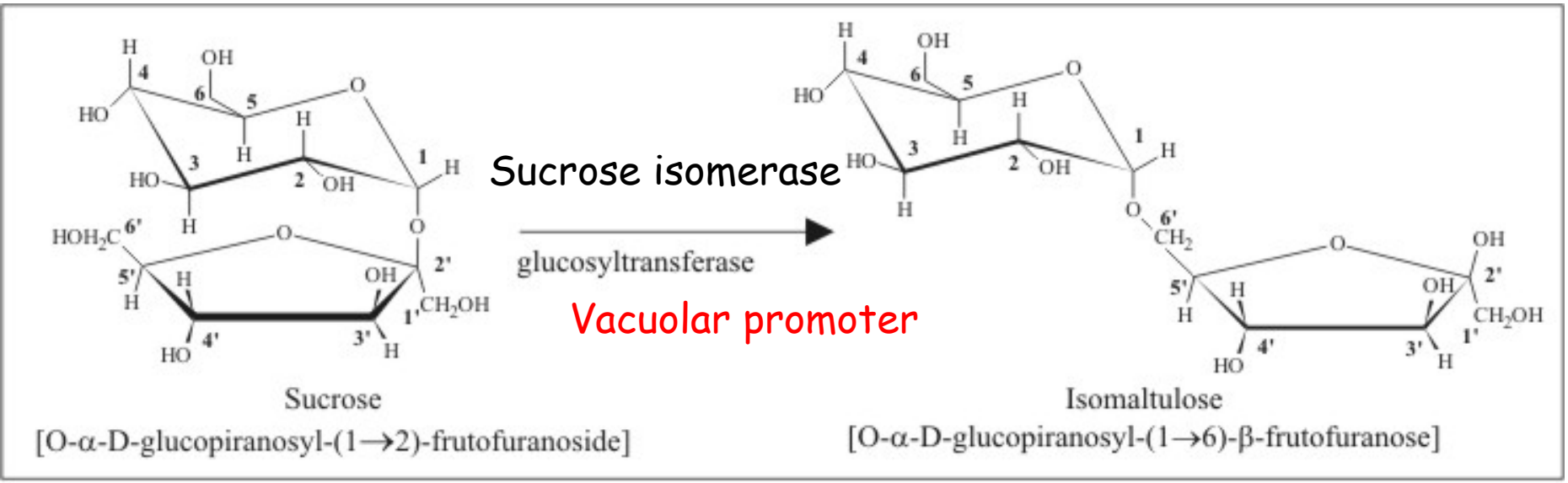


Figure 1. Conversion of sucrose into isomaltulose by glucosyltransferase.

Accumulates as much isomaltulose as sucrose

Sucrose constant = doubled yield

Problem: yeast do not metabolize isomaltulose

Need a gene for yeast - find or shuffle

Oilseed rape is favored for temperate climes

Is it nice to the environment?

Worldwide, oilseed rape emits ca. 9000 Tons

Methyl Bromide*

Before the ban Europe consumed 18,000T of methyl bromide

Is "natural" MeBr ok and synthetic bad?

Is it ok to double the area - for biofuel?

*Gan, J., *et al.* (1998) Production of methyl bromide by terrestrial higher plants. *Geophysical Research Letters* 25, 3595-3598

Brassica has a bifunctional methyl-transferase

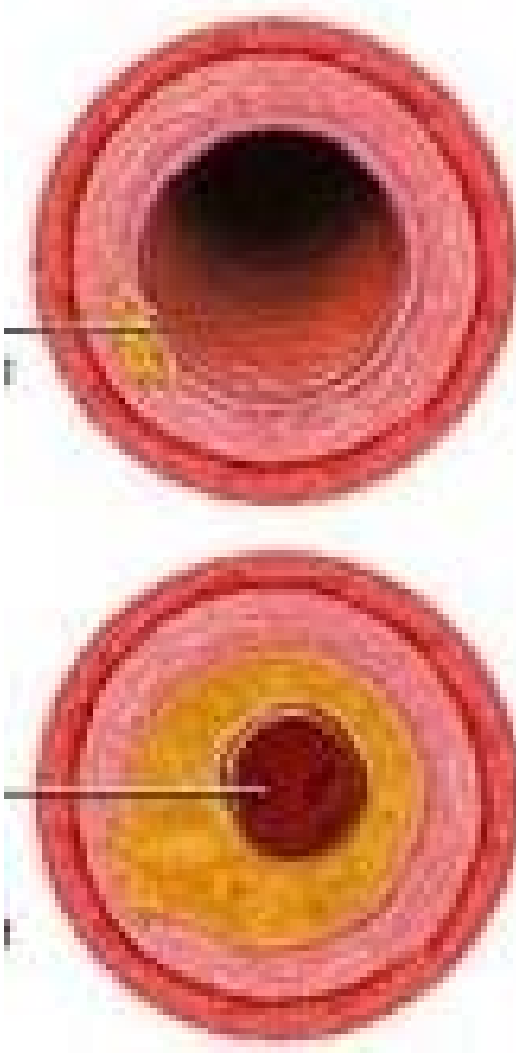
methylates halides to methyl halides (MeBr)
methylates bisulfides to methanethiol
(goes to H_2SO_4 to acid rain)
TDNA disruptive insertion in related *Arabidopsis HOL (harmless to ozone layer)* gene reduced MeBr >99%
To meet intent of methyl bromide ban and reduce acid rain, must cultivate only transgenic oilseed rape with this gene suppressed; non-transgenic should be banned

Palm oil makes poor biodiesel - congeals at low temperatures

Must catalytically crack it - or mix

Needs shorter chain length
- antisense elongases

Needs more mono un-saturation
- engineer desaturases



Such engineering = in non-cholesterogenic "palm-olive" oil

Area needed to replace 15% of USA transport fuels USA

Crop	Oil yield (l/ha)	area needed (M ha)	% of existing arable
Maize	172	462	178
Soybean	446	178	67
Oilseed rape	1,190	67	42
Jatropha	1,892	42	13
Oil palm	5,950	13	7.2
Algae/cyanobacteria[a]	59,000	1.3	1.3
Algae/cyanobacteria[b]	137,000	0.6	0.6

[a]containing 30% oil [b]containing 70% oil

Calculated from Chisti, Biotech. Adv. 25:294-306, 2007

The first generation

not sustainable in medium term

The second generation

Using agricultural wastes

lignocellulosics

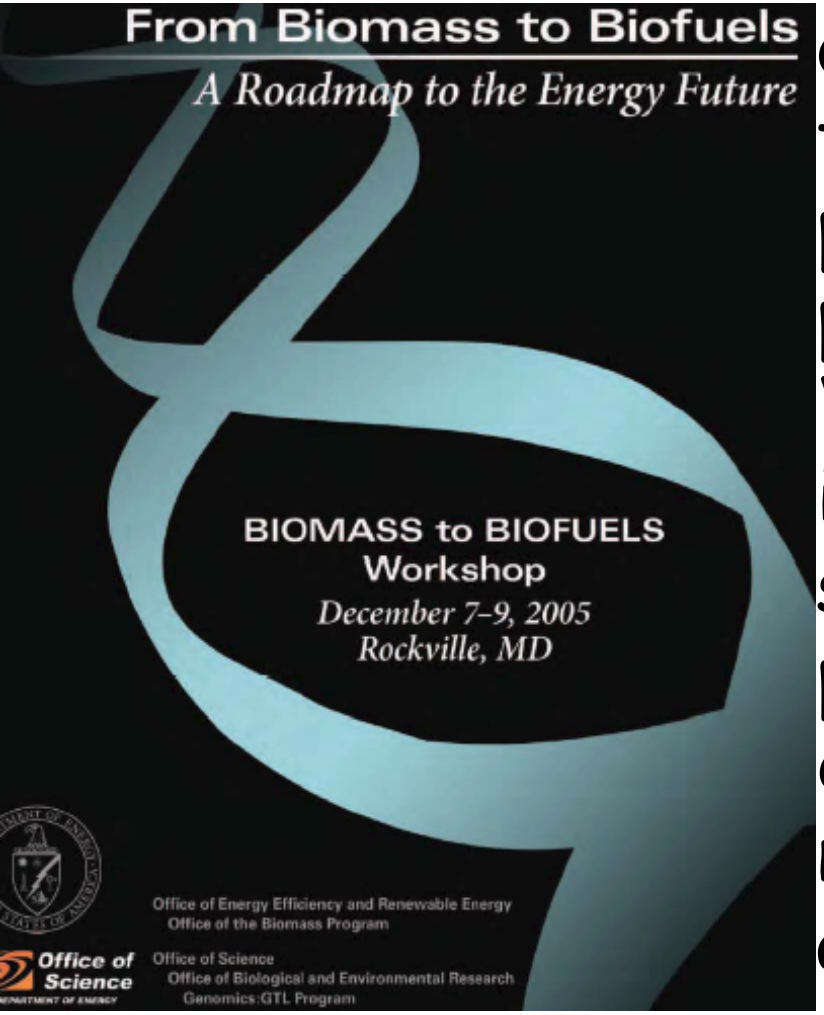
Cultivating biofuel dedicated crops

perennial lignocellulosics

perennial oilseeds

THE DIFFERENCE BETWEEN SCIENCE AND MAGIC

IS THAT MAGICIANS USUALLY KNOW WHAT THEY'RE DOING.



Executive Summary says:
"The key to a new biofuel industry based on conversion of cellulose (and hemicellulose) to ethanol is to understand plant cell wall chemical and physical structures.
With this knowledge, innovative energy crops specifically designed for processing to biofuel can be developed concurrently with new biology-based treatment and conversion methods."

Harvesting perennial Miscanthus

http://www.regensw.co.uk/images/miscanthus_harvesting.jpg

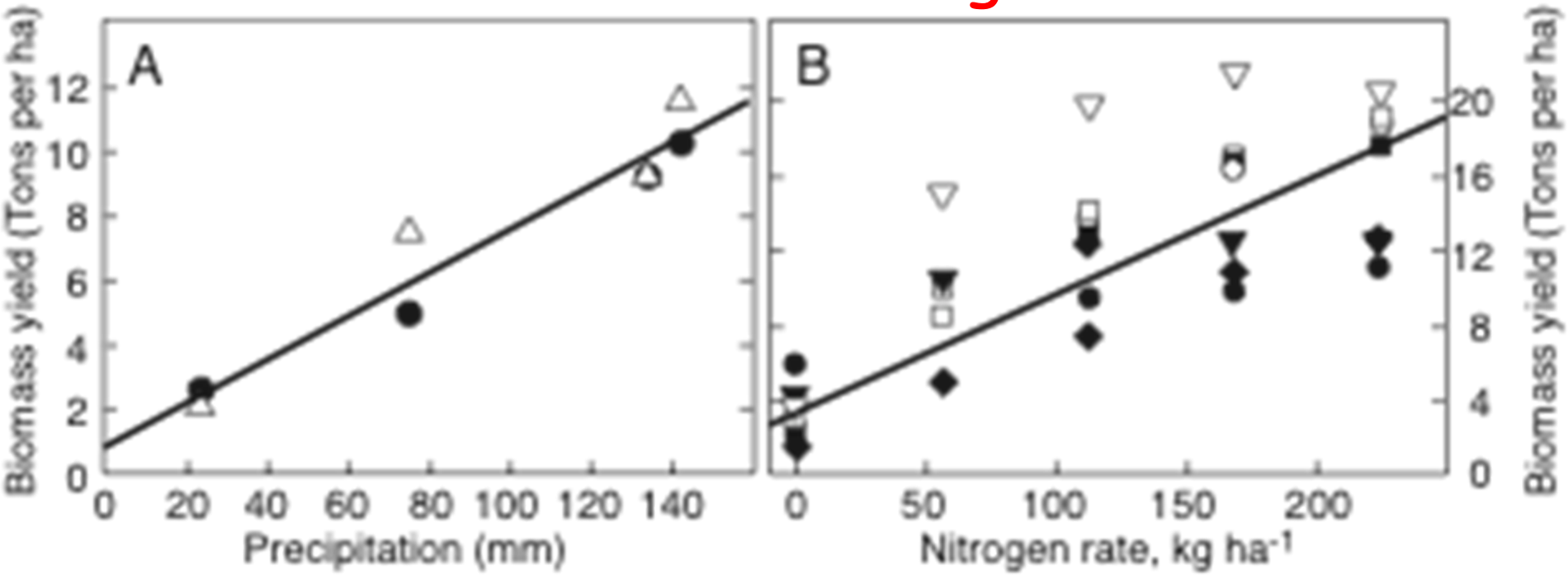
Multi-cut Switchgrass in Italy

www.switchgrass.nl/ photo_gallery.htm

Switchgrass does not defy the law of conservation of matter - grows best with

water

nitrogen fertilizer



Data of Lee et al. and Muir et al, collated in Gressel, "Genetic Glass Ceilings, Hopkins, 2008

The non-degraded switchgrass residue is burnt - energy for process

Contains 5-10% ash, >60% of ash=silica
On burning releases 50% more non-precipitable silica than coal*
Same with sugarcane bagasse/other grasses

*Blevins, L.G., and Cauley, T.H. (2005) Fine particulate formation during switchgrass/coal cofiring. *Journal of Engineering for Gas Turbines and Power-Transactions of the ASME* 127, 457-463

Silicon not a required element for plants

small amounts may be useful

but not the high amounts in many grasses, including sugarcane

Silicon transporters being discovered in plants

antisense/RNAi to lower levels?

With "switchcanthus", land must be bought, dedicated to cultivation, watered, fertilized and harvested.

Straw is available "free" - a byproduct of grain production

World grain production (≈straw production)

wheat	rice	maize	sorghum	millet
million metric tons				
568	579	602	55	26

Total grain (total straw) ≈2,000 million tons

Source: FAO statistics –

Why not use 2 billion T of free waste biomass?

Straw is not good for construction

Straw has negative economic/environmental value

- harbors pathogens if not burnt
  - -requires fungicides on next crop
- releases CO_2 if burnt
- binds nutrients while biodegrading
  - - requires more fertilizer - pollution

Straw has little value as animal feed or as a feedstock for bioethanol production.

- despite ca. 70% carbohydrate
- less than half digested

Sugarcane bagasse = straw in this discussion

Can we turn straw into something valuable?

Maybe not into gold, but into bioethanol

The higher the lignin content the lower the digestibility

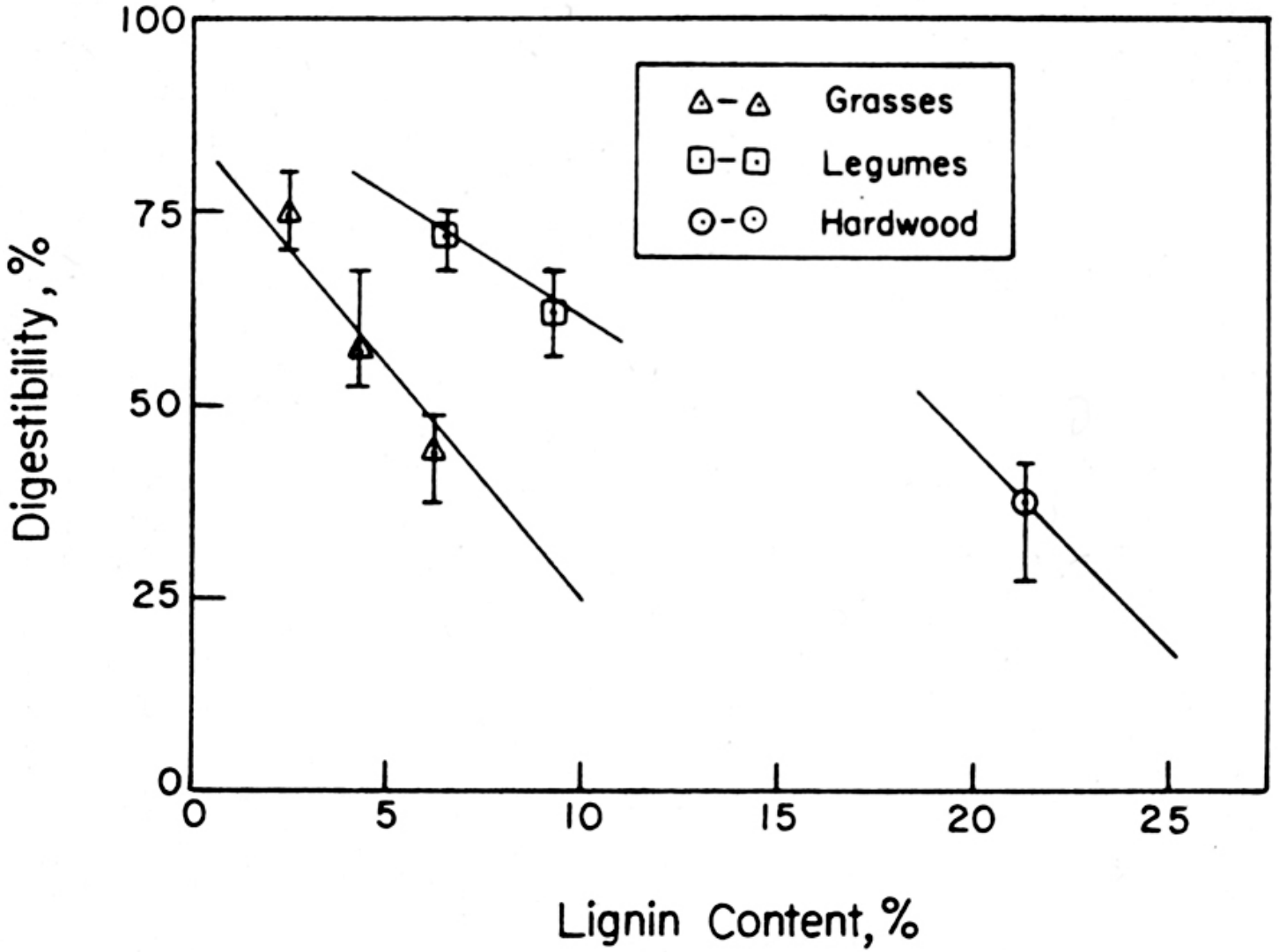


Fig. 6. Relationship between the dry matter digestibility and lignin content

The more lignocellulose is delignified the greater the digestibility by cellulases

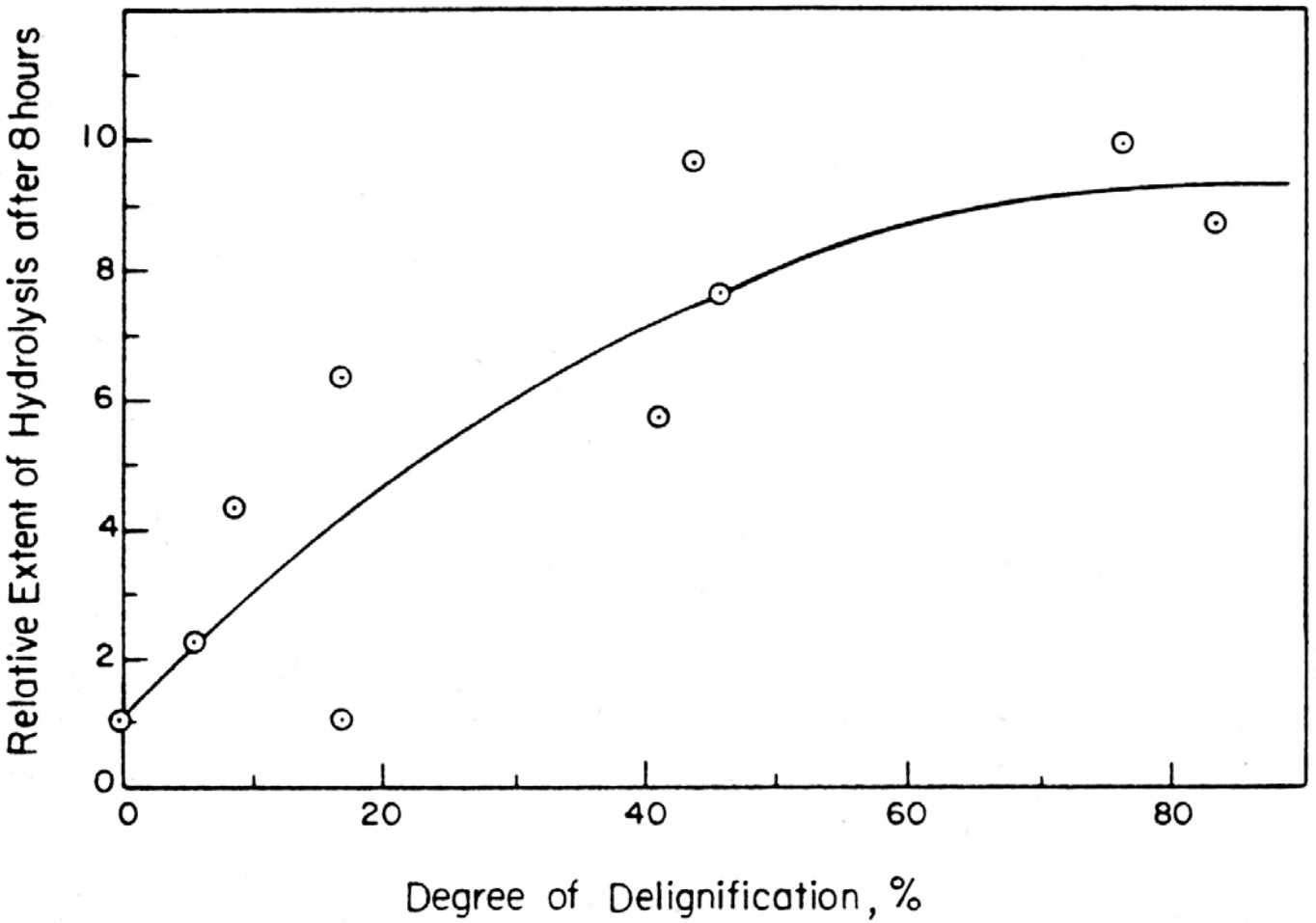
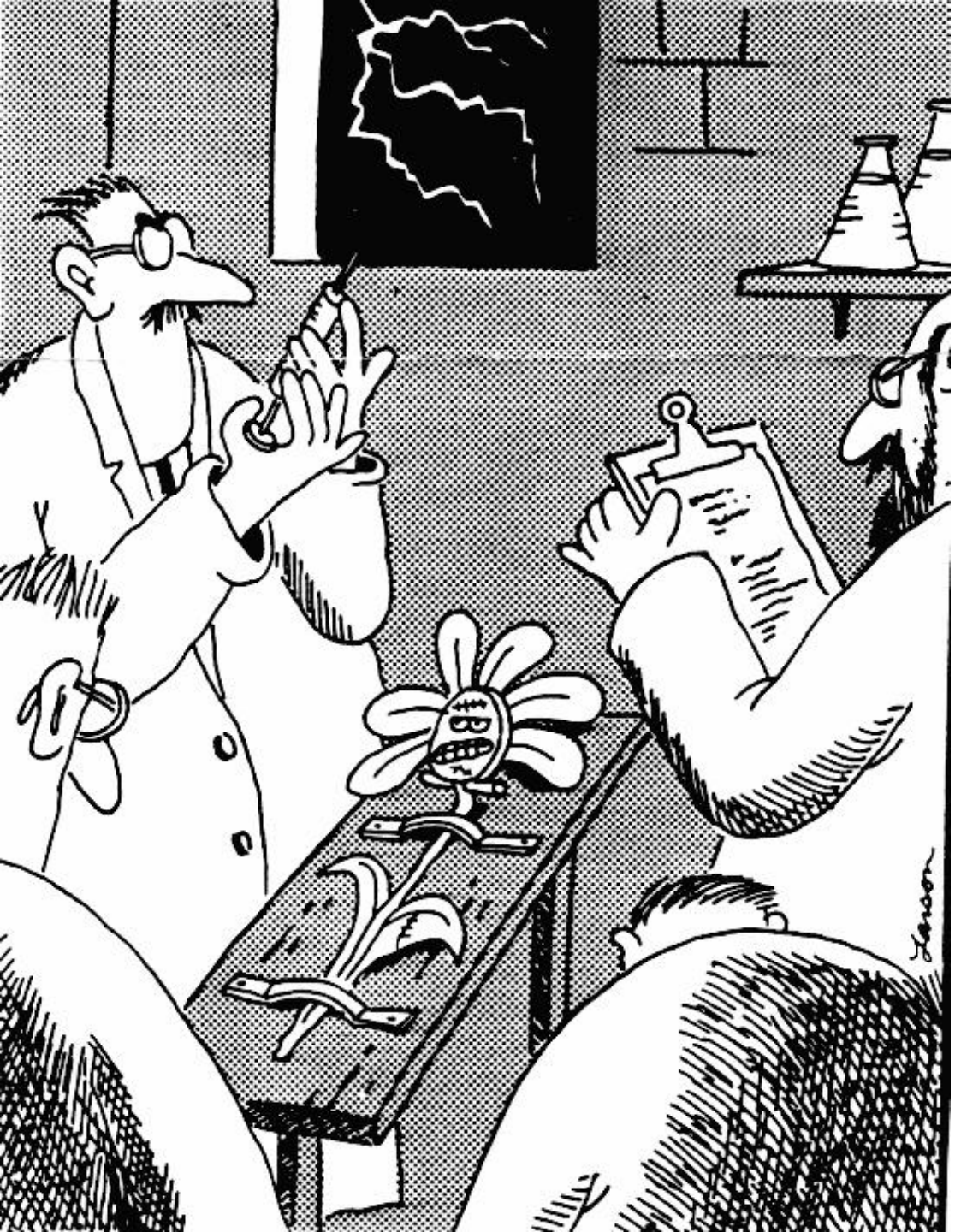


Fig. 5. Relationship between the extent of delignification and the hydrolysis rate



The solution:

Modify straw for:

-less lignin

or

-modified lignin

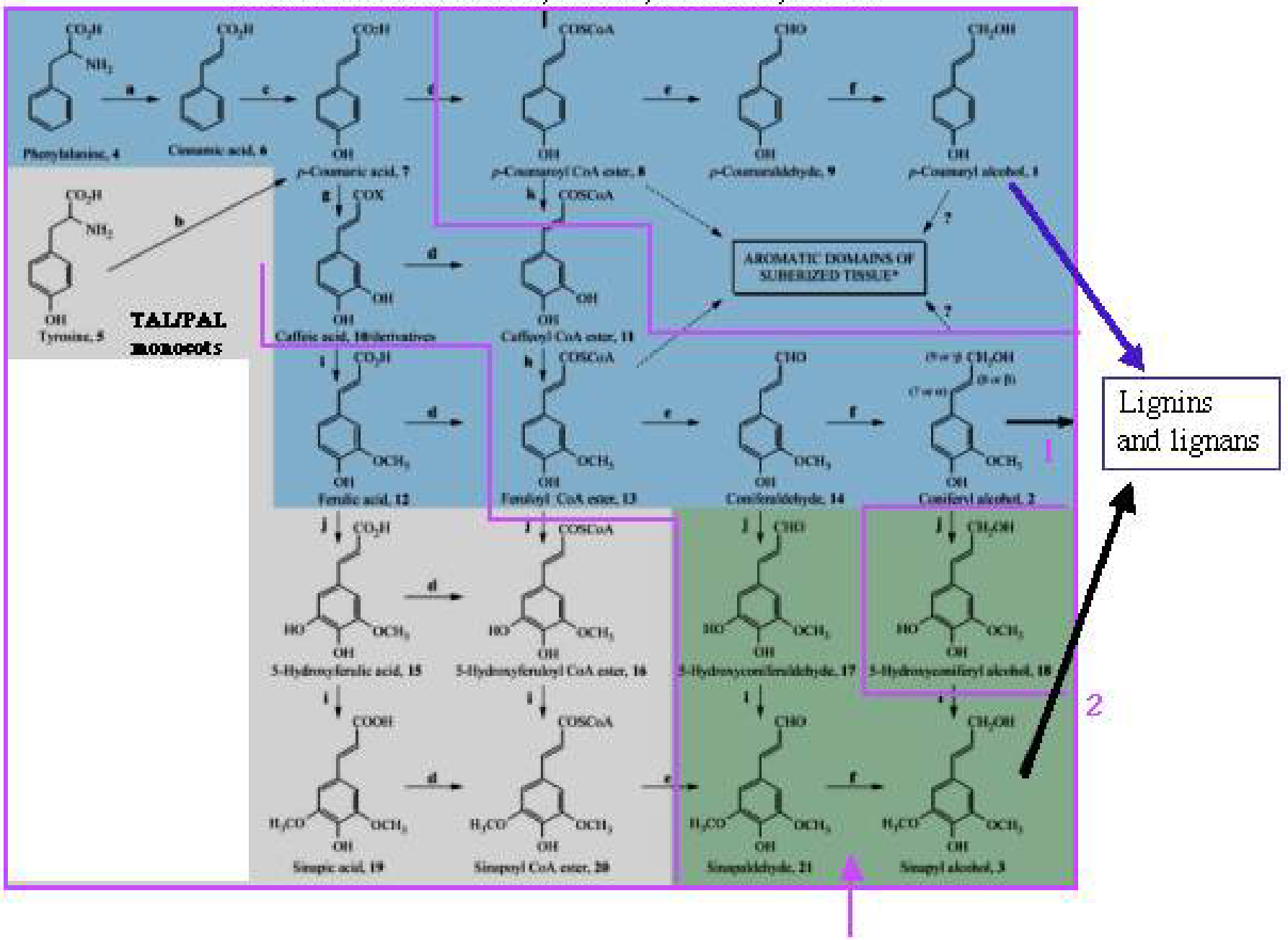
or

- more cellulose

Should reduce the acid/heat requirement, add to yield

Suggested biosynthetic pathways

Anterola and Lewis, 2002; Li et al, 2003



There are many key enzymes/genes known

Rice Gene[a]	type	No. copies identified	Sequence identity (%) barley	wheat	maize	dicot[b]
PAL (AK067801.1)	FL-cDNA	at least3[c]	86	85	86	<76
C4H (AK104994.1)	FL-cDNA	at least 2	89	89	87	<80
C3H (AK099695.1)	FL-cDNA	at least 2	ni	89	79	<80
4CL ((AK105636.1)	FL-cDNA	at least2	83	ni	76	<80
CCoAO (AK065744)	FL-cDNA	at least 3	ni	93	90	<82
F5H (AK067847)	FL-cDNA	at least 2	ni	ni	ni	LS
COMT (AK061859.1)	FL-cDNA	>1	71	86	87	LS
CCR (AK105802)	cDNA	at least 3	88	85	90	<75
CAD (AK 104078)	FL-cDNA	at least 4	ni	ni	83	<71

The sequences are known

Use RNAi or antisense technologies

generate many transformants

will suppress to varying levels

screen optimal suppression/modification

Less lignin should = higher grain yield

Despite common suggestions / myth:

no correlation between lignin and strength

No reason to expect increased lodging if lignin slightly modified and / or reduced by a few percent

More cellulose

Engineer over-production of the cellulose binding domain causes over-production of cellulose

Probably best - stack

lignin reduction/modification

cellulose over-production

Proposal:
Until Malthus arrives in developed world & until CO_2-free fuel sources available, use modified straw in:

Developed world:

Use technology for bioethanol

Developing world:

Use technology for ruminant feed

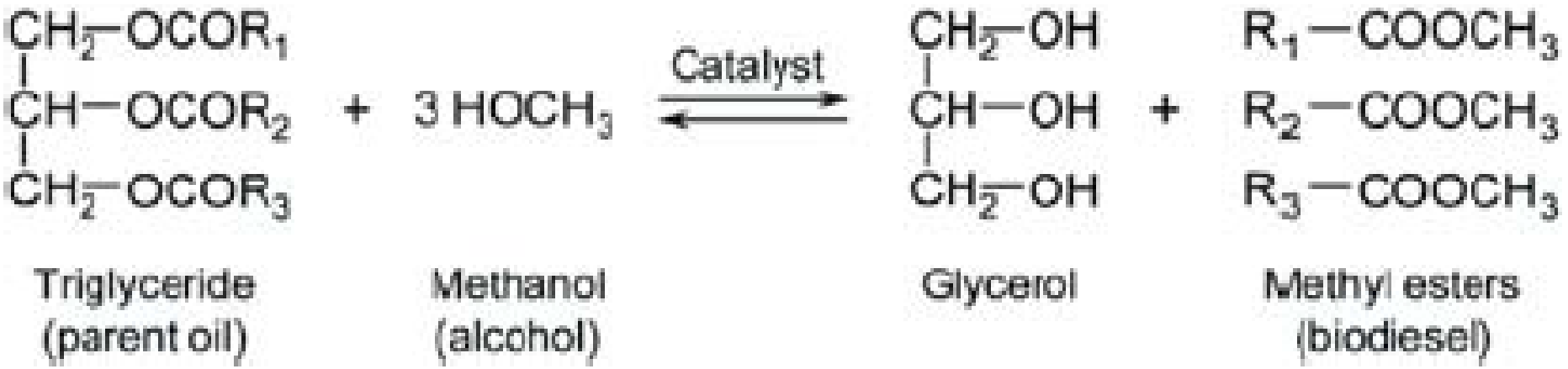
All users should get carbon credits

Is using straw waste sustainable?

Soil scientists say "no!"
-need organic matter in soil
-(but straw used to be burnt in Europe)

Most now agree - ok if 20% left in field

Biodiesel from various sources



Jatropha for biodiesel

www.jatrophaworld.org/

30% oil - seeds get US$140/ton (optimistic)

- fruits hand harvested
- fruits dried in the shade
- seeds removed by hand

Is Jatropha a gimmick to keep the poor poor?

Processing reminiscent of backyard steel mills in China during the cultural revolution

Info not in sites promoting *Jatropha curcas*

common plant names: Black vomit nut, Purge nut, etc.
common oil names: hell oil, oleum infernale, etc.

Toxins: Curcin (a toxalbumin) - similar to ricin
Phorbol esters - diterpenoids Alkaloids
skin tumor promoters

No antidote known

See: http://www.inchem.org/documents/pims/plant/jcurc.htm

Also: A case of Jatropha poisoning resembling organophosphate intoxication Clin. Tox. 44 337,2006

Could one release a transgenic crop with such components? What to do with toxic byproducts?

Is "non-toxic"-Mexican *Jatropha* not toxic?

	curcin[a]	phorbol esters[b]	trypsin inhibitor[c]	phytate[d]	saponins[e]
3 *Jatropha* varieties (average)	102	2.39	20.3	8.9	2.2
"non-toxic"-Mexican *Jatropha*	51	0.11	26.5	8.9	3.4
soybeans (control)	<0.5	-	3.9	1.5	4.7

[a]measured as lectin haemagglutination; [b]mg/g kernal; [c]mg/g meal; [d] % in dry matter meal; [e] measured as % diosgenin equivalents in the meal. Source: Modified from Makkar et al. [18]

18. Makkar, H.P.S., Aderibigbe, A.O. and Becker, K. (1998) Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. Food Chemistry 62, 207-215.

Websites claim "curcin is heat degradable"
Quoted citation says "degradable by prolonged autoclaving"

What to do with toxic byproducts?

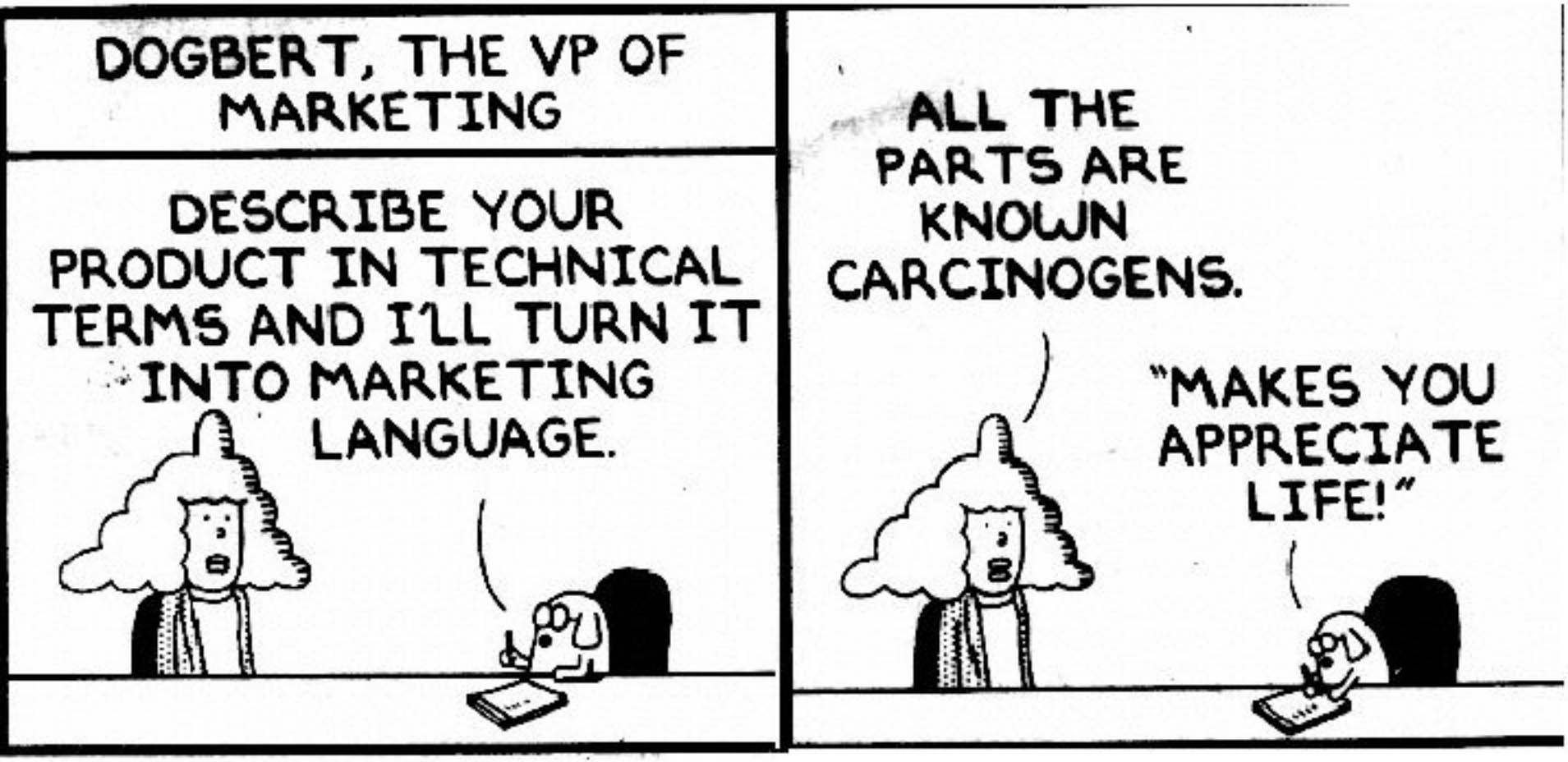
Websites suggest - Use residue as manure

no environmental impact studies

Could one release a transgenic crop with such components? *Jatrofraud! ?*

Remember - with soybeans there is more value from meal than oil….
Where are the economics of discarding "castropha" meal?

Hype for toxic oilseeds



Castor oil for biodiesel

Castor has similar problems as Jatropha

Seeds contain 0.2 to 3% ricin

1 mg/kg toxic
fill car with 50 liters (13 gallons diesel)
enough ricin coproduct to kill 3 people
at lowest content, 45 at highest

Not transgenic - no environmental impact studies needed - no regulatory scrutiny

Ricin protein "easy" to eliminate transgenically!

Can reduce by breeding
- why not continue breeding?

Ricin production dominant
pollen from neighbors

RNAi/antisense dominant for non-production

Override pollen

If you want "Castropha" as an oil crop - engineer or breed:

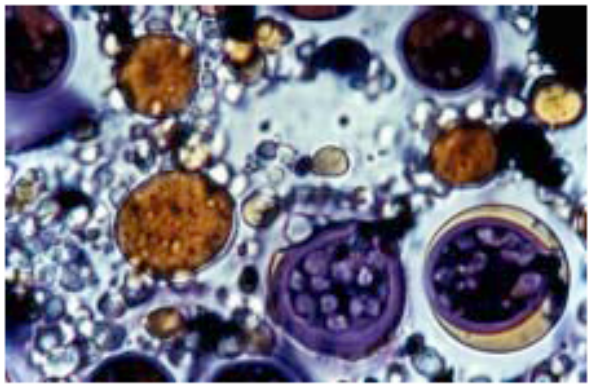
- dwarfing (increase harvest index)
- single stalk (high IAA?)
- Antishattering - fruits dry on stems
  -machine harvesting and threshing
- RNAi curcin /ricin & agglutnin genes
- RNAi terpene synthase to rid of phorbols
- RNAi pathways to other toxins/allergens
- better yield, oil content / quality

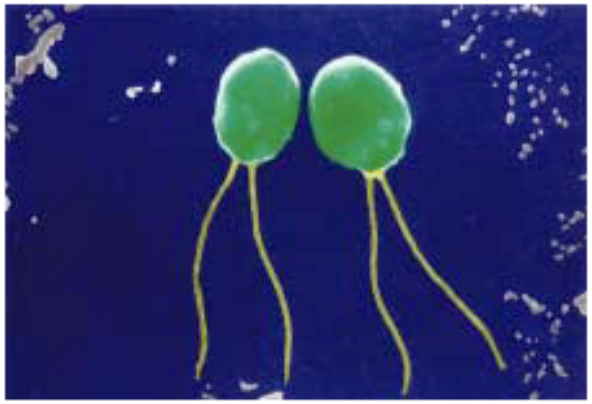
The engineered crop might then be safe to grow

National Renewable Energy Laboratory

NREL/TP-580-24190

A Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae

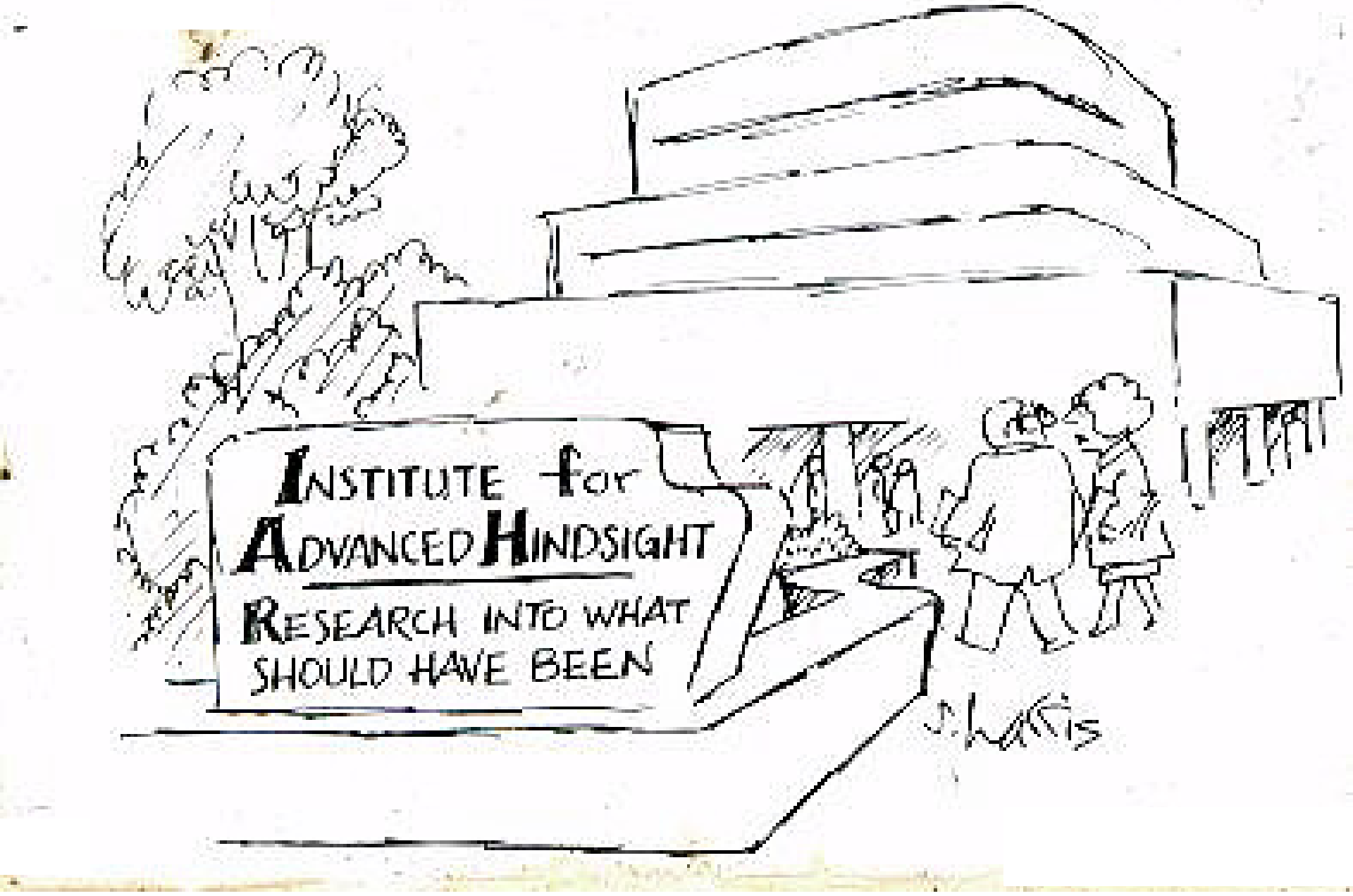




Close-Out Report

1978-1996 DOE Projects
Closed because breakeven only at $70/barrel petroleum

Let us visit the



Asked: is growing algae for biofuel feasible?

Immediate answer – no, undomesticated

Read the DOE report

- thought what genes are needed

Wrote a report summarizing ideas

Problems to be solved

Choice of organisms – algae or cyanobacteria
Contamination by unwanted organisms
Wastage of light energy
Cooling
Unneeded proteins
Needed co-product proteins
Oil content
If transgenic - spillage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